

AMENDMENTS TO THE SPECIFICATION

Please replace the prior filed Sequence Listing with the substitute Sequence Listing, filed herewith.

Please insert the following new paragraph at page 1 of the specification, following the title:

This application claims priority under 35 U.S.C. §119(a)-(d) to Chinese patent application serial number 00119553.0, filed on August 2, 2000 and Chinese patent application serial number 001103102.6, filed on January 18, 2001.

Please replace the paragraph beginning at page 1, line 19 of the specification with the following amended paragraph:

Trichosanthin (TCS) is originally isolated from the root tuber of a Chinese medicinal herb *Trichosanthes kirilowii* Maximowicz and is identified as the active component of Tian Hua Fen, a Chinese medicine described and used clinically as early as two thousand years ago. Chemically, TCS is a 27 kDa sized type I ribosome-inactivating protein (RIP). It possesses RNA-N-glycosidase activity that inactivates the 60S subunits of the eukaryotic ribosomes (Zhang JS, Liu WY, The Mechanism of Action Trichosanthin on Eukaryotic Ribosome RNA N-glycosidase of the Cytotoxin, *Nucleic Acids Res*, 20 (6): 1271-1275, 1992). Native TCS is composed of 247 amino acid residues. Its primary structure is shown in Fig. 1 (Nie HL, et al., The Cloning and Structural Analysis of Trichosanthin Gene, The 4th China Conference on Gene Structure Cloning and Expression, Haikou, A-23, 1991). TCS has been used clinically in China since the 1970's to induce mid-term abortion and to treat diseases of trophoblastic origin, e.g., hydatiform mole (Second Research Group of Shanghai Institute of Experimental Biology, *Science in China*, 19: 811-830, 1976). Soon after the laboratory finding in 1989 that TCS appeared to inhibit the HIV-1 replication in both acutely infected T-lymphoblastoid cells and in chronically infected macrophages (McGrath MS, Hwang KM, Caldwell SE, et al., GLQ233: An Inhibitor of Human Immunodeficiency Virus Replication in Acutely and Chronically Infected Cells of Lymphocyte and Mononuclear Phagocyte Lineage, *Proc. Natl. Acad. Sci. USA*, 86: 2844-2848, 1989), clinical trials of TCS as a potential treatment for AIDS

were carried out. In addition to HIV, TCS is capable of attacking other types of virus. It was also found toxic to leukemia cells and other types of tumor cells (Kong M, Ke YB, Zhou MY, et al., Study on Trichosanthin Induced Apoptosis of Leukemia K562 Cells, *Acta Biologiae Experimentalis Sinica*, 31 (3): 233-243, 1998; Zheng YT, Zhang KL, Ben KL, et al., In Vitro Immunotoxicity and Cytotoxicity of Trichosanthin Against Human Normal Immunocytes and Leukemia-lymphoma Cells, *Immunopharmacology and Immunotoxicology*, 17 (1): 69-79, 1995; Wu YX, Xiang DN, Zhang SP, et al., The Toxic Effect and Its Mechanism of Trichosanthin Against Stomach and Colon Cancer Cells, *Chinese Journal of Digestion*, 13 (3): 263-266, 1993). In clinical uses, however, a dangerous complication was observed with the drug. It can occasionally cause immediate type allergic reaction mediated by immunoglobulin E (IgE) antibody. The TCS specific IgE reacts to TCS in the body, initiating the onset of type I hypersensitivity manifested clinically as complications such as allergic urticaria, angioedema, and anaphylactic shock - a sudden, severe, life-threatening allergic reaction that can kill within minutes. This dysfunctional immune response to TCS usually remains strong [[positive]] in the recipient's body for many years. As a result, TCS is restricted to only one administration during the recipient's lifetime as an abortifacient. Not only in abortion, allergic reactions were also present when TCS was used in treating other diseases. Its application was therefore greatly restricted.

Please replace the paragraph beginning at page 5, line 10 of the specification with the following amended paragraph:

FIG. 1 presents the amino acid sequence of the native TCS (SEQ ID NO.1 in the Sequence Listing) and the nucleotide sequence coding the same (SEQ ID NO.2). The region of positions 1 through 247 shows the amino acid sequence of the mature native TCS. The mature native TCS (amino acids 1 through 247) are also set forth as SEQ ID NO:8.

Please replace the paragraph beginning at page 5, line 13 of the specification with the following amended paragraph:

The present inventors have discovered after extensive studies that the immunological reactive regions of TCS are structurally located at amino acid residues between 174 to 180, 203 to 226, and 230 to 244. The modification of at least one amino acid residue within these three regions can produce a novel MTCS protein with excellent properties. The antigenicity of TCS is largely reduced in the MTCS while the biological activities of TCS being substantially retained in the MTCS. A MTCS according to the present invention retains at least RIP activity and abortifacient activity, and preferably retains all of the biological activities including anti-tumor and anti-virus activities[[], etc]].

Please replace the paragraph beginning at page 6, line 1 of the specification with the following amended paragraph:

The numerical positions of amino acid residues mentioned in the description and claims of this application refer to the residue numbers as shown in Fig.1 and SEQ ID NO:8.

Please replace the paragraph beginning at page 11, line 31 of the specification with the following amended paragraph:

The DNA sequence encoding MTCS obtained from site-directed mutagenesis can be digested by appropriate restriction enzyme, e.g., NcoI (or NdeI) and BamHI, and then cloned into an expression vector e.g., plasmid vector pET-2d (or pET-3a). The resultant mutant expression vector can be used to transform an appropriate host cell, e.g., E. Coli BL21 (DE3, pLysS) following standard procedures. The transformant is then cultured in appropriate medium. Inducer, e.g., isopropyl-beta-D-thio-galactoside (IPTG) can be added to the culture at a proper time, if necessary, to induce expression of MTCS, which can then be collected from the culture with standard methods including lysing the host cells, centrifuging the lysate, and purification by column chromatography[[], etc]].

Please replace the paragraph beginning at page 15, line 8 of the specification with the following amended paragraph:

For a TCS deleting mutant, when 3 amino acid residues are deleted from the C terminus, its in vitro reactivity with immunoglobulin G (IgG) and IgE remains as strong positive as native TCS. Its immunological reactivity is reduced when the deletion is increased to 5 amino acid residues, while in this case no effect on its RIP activity ~~[[and]]~~ or abortifacient activity is observed. Not until 29 amino acid residues are deleted is there a decrease in its biological activities. It was reported by the present ~~[[inventers]]~~ inventors that the biological active center of TCS is located at positions between 110-174 (Ke YB, Chen JK, Nie HL, et al., Structure-function Relationship of Trichosanthin, Life Sciences, 60 (7): 465-472, 1997). The three-dimensional structural change caused by deletion of C-terminal sequence also affects this active center. The closer the deleted sequence to the active center, the more the reduction of biological activities. It is now discovered that the immunological reactive region is structurally located closer to the C-terminus than the biological active center. Consequently it is more easily affected by deletion of C-terminal sequence. The modifying mutants M7TCS(1-247)[one of the amino acid residues between positions 174 to 180 is mutated], M24TCS(1-247) [one of the amino acid residues between positions 203 to 226 is mutated] and M15TCS(1-247) [one of the amino acid residues between positions 230 to 244 is mutated] in Table 4 demonstrate weak in vitro reactivities with IgG and IgE, and potent biologic activities including RIP and abortifacient activities. This result indicates that the three modified regions of amino acid sequence (positions 180-174, 226-203, 244-230) are relevant with the immunological reactivities. Any structural changes in these regions may cause the reduction of the immunological reactivities. These structural changes include the deletion of at least one amino acid residue; the insertion of at least one amino acid residue between two adjacent amino acid residues; the addition of at least one amino acid residue to these sequences; the replacement of at least one hydrophilic amino acid residue with a hydrophobic amino acid residue; the replacement of at least one hydrophobic amino acid residue with a hydrophilic amino acid residue; the replacement of at least one acidic amino acid residue with a basic amino acid residue; the replacement of at least one basic amino acid residue with an acidic amino acid residue; the coupling of at least one amino acid

residue to a chemical entity; and/or any modification including insertion of at least one amino acid residue that can cause a change in the electric charge of the amino acid site where the same modification is being made. The modifying mutants M31TCS(1-247) [one of the amino acid residues between positions 174 to 180 and one of the amino acid residues between positions 203 to 226 are mutated] and M46TCS(1-247) [one of the amino acid residues between positions 174 to 180, one of the amino acid residues between positions 203 to 226 and one of the amino acid residues between positions 230 to 244 are mutated] demonstrate negative immunological reactivities and potent biological activities including RIP and abortifacient activities. These two are the mutants of excellent properties. The antigenicity of native TCS is largely reduced. But the biological activities of native TCS are retained. The antigenicity of M46TCS(1-247) is even lower compared to M31TCS(1-247).